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PII: S0960-894X(14)00456-9
DOI: http://dx.doi.org/10.1016/j.bmcl.2014.04.099
Reference: BMCL 21592

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 17 January 2014
Revised Date: 7 April 2014
Accepted Date: 23 April 2014

Please cite this article as: Dhumaskar, K.L., NandanMeena, S., Ghadi, S.C., Tilve, S.G., Graphite catalyzed solvent free synthesis of dihydropyrimidin-2(1*H*)-ones/thiones and their antidiabetic activity, *Bioorganic & Medicinal Chemistry Letters* (2014), doi: http://dx.doi.org/10.1016/j.bmcl.2014.04.099

Graphical Abstract

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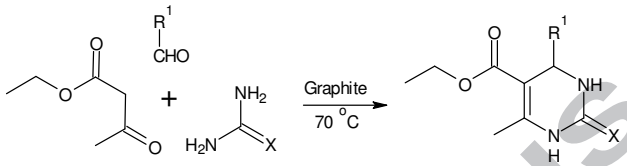

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Biginelli reaction
Multicomponent reaction
Solvent less
Graphite catalysis
Dihydropyrimidinones
α-amylase inhibition
anti diabetic.

ABSTRACT

A solvent free three component condensation reaction between an aldehyde, ethyl acetoacetate and urea catalyzed by graphite, a green catalyst is described for the synthesis of dihydropyrimidin-2(1*H*)-ones. This protocol is scalable and the catalyst is reusable. This method is also applied for the synthesis of dihropyrimidin-2(1*H*)-thiones. α-amylase, a key enzyme in carbohydrate metabolism is generally targeted for management of type 2 diabetes. The therapeutic potential of the dihydropyrimidinones and dihropyrimidinthiones to inhibit α-amylase activity was evaluated by *in vitro* assay. Of the synthesized compounds 3,4-dihropyrimidin-2(1*H*)-thione (**1k**) demonstrated highest inhibition of α-amylase activity.

Type 2 diabetes is the most predominant form of diabetes mellitus resulting from insulin resistance and relative insulin deficiency.[1] α-Amylase, a key carbohydrate digestive enzyme hydrolyses dietary starch and is responsible for augmentation of postprandial glucose. The failure to control the postprandial glucose levels leads to long term health complications such as heart disease, diabetic retinopathy and strokes. Modulation of α-amylase activity using synthetic inhibitors is considered a promising and effective therapeutic for management of hyperglycemia.[2] The stabilization of glucose level would preclude hyperglycemia and ward off type-2 diabetes associated health complications.[3] Thus efforts are being pursued to test the natural and non-natural compounds for antidiabetic activity.

Acarbose a natural antidiabetic drug that demonstrates inhibition of pancreatic α-amylase activity is being marketed successfully to control postprandial glucose.[4] Additionally, during *in vitro* evaluation and testing of candidate drugs with plausible α-amylase inhibitory activity, acarbose has been frequently used as a positive reference standard.[5]

Dihydropyrimidinones (DHPMs) are known for their broad spectrum of biological activities such as antifungal, antibacterial, anti-tubercular, anti-inflammatory antioxidant, anti HIV, anticancer, anti-malarial, anti-filarial,[6] and are additionally used as building blocks for the synthesis of various heterocycles.[7] The three component reaction between an aldehyde, *β*-keto ester and urea or thiourea to give 3,4-dihydropyrimidin-2(1*H*)-ones and 3,4-dihropyrimidin-2(1*H*)-thiones is known as Biginelli reaction.[8] Recently, syntheses of these heterocycles have been comprehensively reviewed.[9] Several catalysts have been successfully employed for this reaction. Recent reports include sulfated tungstate,[10] periodic mesoporous organosilica,[11] silica gel supported polyphosphoric acid (PPA-SiO_2),[12] titanium silicate,[13] Iron(III) tosylate,[14] BF_3,[15] $FeCl_3$,[16] $InCl_3$,[17] $Yb(OTf)_3$,[18] ionic liquid,[19] t-$(CH_3)_3COK$,[20] Ph_3P,[21] and L-proline.[22]

Graphite, an allotrope of carbon, though much known as support for organic reagents is less used as a reagent itself. The very few applications of graphite as catalyst are Friedel-Craft alkylation,[23] acylation reaction,[24] Diels Alder reaction,[25] and for conversion of aldehydes into nitriles.[26] To further its use, recently we used it successfully for the synthesis of quinoxalines.[27] Continuation of these studies herein we report graphite as an inexpensive heterogeneous, nontoxic, recyclable green catalyst under solvent free conditions for the synthesis of DHPMs.

In our efforts, initially we tried the reaction of benzaldehyde, ethyl acetoacetate and urea in ethanol on 1mmol scale. Checking

(TLC) that no reaction takes place by stirring the reaction mixture we added 100 mg of graphite (flakes, Aldrich chemicals) and continued stirring the reaction mixture further. However after prolonged stirring we could not notice (TLC) any product formation. Next, we tried the reaction in other solvents like dioxane, chloroform, toluene, THF and methanol in presence of graphite. In none of these cases we could observe (TLC) any product formation. Further, we attempted the reaction neat without any success. Next we heated the reaction mixture containing graphite on an oil bath at three different temperatures (50, 60 & 70 °C). Although no product formation was observed for the reactions at 50 and 60 °C, we could see solid product separating out and reaction completing in one hour for the reaction at 70 °C. The product was obtained in 90% yield. In a recent report using TiO_2 as a catalyst, it is mentioned that the reaction does not work at 70°C without the use of catalyst.[28] In another report, sulfonated carbon[29] is used as a catalyst at 140 °C under solvent free conditions. Having confirmed that product DHPM can be obtained at 70°C using graphite we further studied the optimization of catalyst loading. After several experiments with diminishing quantities of graphite, 10% w/w was found to be sufficient to complete the reaction in a reasonable amount of time. With optimized reaction conditions, we further extended this method for a wide variety of aryl aldehydes carrying electron withdrawing or electron donating groups, at *ortho*, *meta* or *para* position **1a-1j** (Table 1). In general it was observed that aryl aldehydes having electron donating group reacts slower compared to aryl aldehydes having electron withdrawing groups. Thiourea also reacted in a similar manner to give the corresponding 3,4-dihropyrimidin-2(1*H*)-thiones **1k-1n** (Table 1). However in this case we had to heat the reaction mixture at 120 °C. Having successfully obtained the DHPMs, we then investigated the recyclability of our catalyst. For this purpose, the reaction mass was dissolved in ethanol, filtered and recovered catalyst was used for further trials. We could successfully use the recycled graphite for 10 cycles without any appreciable decrease in yield or increase in reaction time. The reaction was also studied for scale up studies up to 10 grams without any problem. The mechanism by which graphite acts as a catalyst is not clearly understood. It may act as a supporting material for adsorption of the of the substrate and delivers the heat rapidly and also acts as a Lewis base (supplementary information).

The dihydropyrimidinones and dihydropyrimidinthiones synthesized during present studies were tested for their *in vitro* inhibition of α amylase activity. They were compared to acarbose which demonstrated 100 % inhibition at 100 mg/mL (Fig.1). The parent DHPM with unsubstituted benzene ring did not display any inhibition of α-amylase activity. Also with electron donating groups on the benzene ring of DHPMs showed no inhibition of α-amylase activity. However, presence of electron withdrawing groups on benzene ring demonstrated α-amylase inhibition, e.g. nitro group at *meta* position (**1f**) displayed 26.49 % inhibition. Further, two compounds from the synthesized series with sulfur in the pyrimidinone ring demonstratedα amylase inhibition. Compound (**1n**) having *para*-methoxy group in the benzene ring displayed 66.5 % inhibition, whereas the parent 3,4-dihropyrimidin-2(1*H*)-thione (**1k**) demonstrated a dose dependent inhibition with maximum inhibition of α amylase activity of 97.2 % at 300µg/mL (Fig. 2).

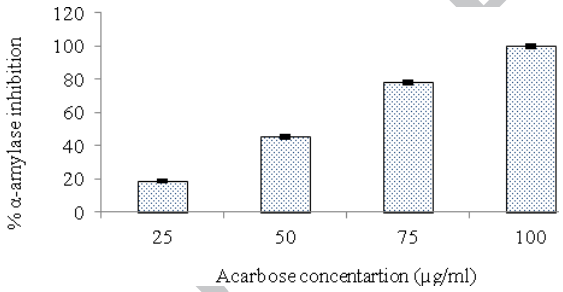


Fig 1. Inhibition of α amylase activity by acarbose at various concentrations

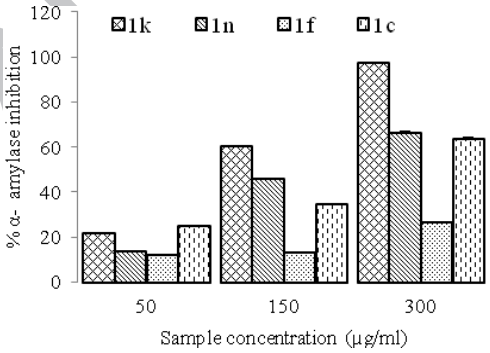
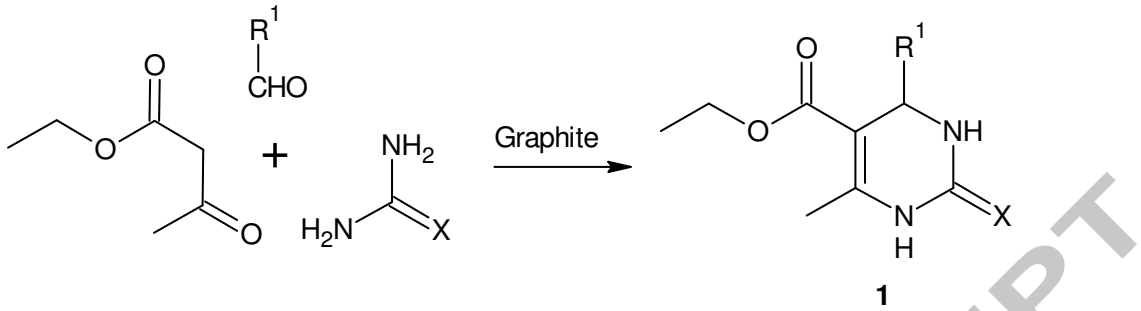


Fig 2. Dose dependent inhibition of α-amylase activity by various dihydropyrimidinones derivatives

Table 1. Synthesis of substituted 3,4-dihydropyrimidinones (**1**) and their anti diabetic activity.



1

1	R^1	X	M.P. (°C)	Temp. (°C)	Time	yield[a](%)	% inhibition of α-amylase activity
a	Ph	O	200-202 (203-205)[30]	70	1h	97	0
b	*p*-ClC_6H_4	O	212-213 (210-212)[30]	70	2h	97	0
c	*p*-FC_6H_4	O	184-186 (184-186)[31]	70	2h	98	63.8
d	*o*-FC_6H_4	O	238-239 (240-241)[32]	70	2h	97	0
e	*p*-$NO_2C_6H_4$	O	195-200 (207-208)[30]	70	2h	91	0
f	*m*-$NO_2C_6H_4$	O	225-226 (226-227)[31,32]	70	2h	96	26.5
g	*p*-$OMeC_6H_4$	O	202-203 (202-203)[30]	70	2h	96	0
h	*m*-$OMeC_6H_4$	O	206-208 (207-208)[31]	70	1.5h	96	0
i	3,4-$OMeC_6H_3$	O	176-178 (176-178)[33]	70	1h	95	0
j		O	188-190 (188-189)[33]	70	1h	96	0
k	Ph	S	205-206 (205-207)[30]	120	10min	96	97.2
l	*m*-HOC_6H_4	S	183-185(184-186)[33]	120	10min	94	0
m	*m*-$MeOC_6H_4$	S	150-153 (150-152)[34]	120	10min	98	0
n	*p*-$MeOC_6H_4$	S	149-151 (150-152)[30]	120	10min	97	66.5

[a]Isolated yields.

In conclusion, we have developed a convenient metal free graphite catalyzed green method for the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones and 3,4-dihropyrimidin-2(1*H*)-thiones. The synthesized compounds were tested for their invitroantidiabetic activity with acarbose as standard. 3,4-dihropyrimidin-2(1*H*)-thione displayed 97.2 % activity at 300µg/mL.

Acknowledgments

We are thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi for project funding. KD is also thankful to CSIR for awarding CSIR-NET (National Eligibility Test) Senior Research fellowship.SNM acknowledges Department of Biotechnology, New Delhi, for award of DBT-SRF.

References and notes

1. Eichler, H. G.; Korn, A.; Gasic, S.; Pirson,W.; Businger, J. *Diabetologia* **1984**, 26, 278.
2. Rhabaso – Lhoret, R.; Chiasson J.L. In *α – Glucosidase inhibitors*; Defronzo, R. A.; Ferrannini, E.; Keen, H.; Zimmet, P., Ed.; *International Textbook of Diabetes Mellitus* Vol. 1, 3rd. John Wiley & Sons Ltd., UK, 2004; pp. 901-914.
3. Mai, T. T.; Chuyen, N. V. *Biosci. Biotecnol. Biochem.* **2007,** 71, 69.
4. Brzozowski, A. M.; Davies, G. J. *Biochemistry* **1997,** 36, 10837.
5. (a) Manaharana, T.; Appleton, D.; Cheng, H. M.; Palanisamy, U. D. *Food Chem.* **2012**, 132, 1802.
   (b) Olaokun, O. O.; McGaw, L. J.; Eloff, J. N.; Naidoo, V. *Bmc Complem. Altern. M.* **2013**, 13, 94.
6. (a) Kappe, C. O.; *Eur. J. Med. Chem.* **2000,** 35, 1043.
   (b) Singh, K.; Arora, D.; Singh, K.; Singh, S. *Mini-Rev. Med. Chem.* 1, **2009,** 95-106.
   (c) Singh, T. P.; Singh, O. M. In *Synthesis and bioactivity of dihydropyrimidines*; Ameta, K. L.; Pawar, R. P.; Domb, A. J., Ed.; Nova Science Publishers; New York, **2012**; pp 117-135.
7. (a) Kim. S. S.; Choi, B. S.; Lee, J. H.; Lee, K. K.; Lee, T. H.; Kim, Y. H.; Hyunik, S. *Synlett* **2009,** 599.
   (b) Yamamoto, K.; Chen, Y. G.; Buono, F. G. *Org. Lett.* 7, **2005,** 4673.
   (c) Wang, X-C.; Quan, Z. J.; Zhang, Z. *Tetrahedron* 63, **2007,** 8227.
   (d) Pérez, R.; Beryozkina, T.; Zbruyev, O. I.; Haas, W.; Kappe, C. O. *J. Comb. Chem.* 4, **2004,** 501.
   (e) Lengar, A.; Kappe, C. O. *Org. Lett.* 6, **2004,** 771.
8. Biginelli, P. *Gazz. Chim. Ital.* 23, **1893,** 360-413.
9. Wan, J-P.; Liu, Y. *Synthesis* **2010,** 3943.
10. Salim, S. D.; Akamanchi, K. G. *Catal. Commun.* 12, **2011,** 1153.
11. Hankari, S. E.; Motos-Pérez, B.; Hesemann, P.; Bouhaoussb, A.; Moreaua, J. J. E. *Chem. Commun.* 47, **2011,** 6704.
12. Dastmalbaf, M. Z.; Davoodnia, A.; Heravi, M. M.; Hoseini, N. T.; Khojastehnezhad, A.; Zamani, H. A. *Bull Korean Chem. Soc.* 32, **2011,** 656.
13. Kulkarni, M. G.; Chavhan, S. W.; Shindhe, M. P.; Gaikwad, D. D.; Borhade, A. S.; Dhondge, A. P.; Shaikh, Y. B.; Ningdale, V. B.; Desai, M. P.; Birhade, D. R. *Beil. J. Org. Chem.* 5, **2009,** 4.
14. Starcevich, J. T.; Laughlin, J. T.; Mohan. R. S. *Tetrahedron Lett.*54, **2013,** 983.
15. Hu, E. H.; Sidler, D. R.; Dolling, U.-H. *J. Org. Chem.* 63, **1998**, 3454.
16. Lu, J.; Ma, H. *Synlett* 2000, 63
17. Ranu, B. C.; Hajra, A.; Jana, U. *J. Org. Chem.* 65, **2000**, 6270.
18. Ma, Y.; Qian, C.; Wang, L.; Yang, M. *J. Org. Chem.* 65, **2000**, 3864.
19. Peng, J.; Deng, Y. *Tetrahedron Lett.* 42, **2001**, 5917.
20. Shen, Z.-L.; Xu, X.-P.; Ji, S.-J. *J. Org. Chem.* 75, **2010**, 1162.
21. Debache, A.; Amimour, M.; Belfaitah, A.; Rhouati, S.; Carboni, B. *Tetrahedron Lett.* 49, 2008, 6119.
22. Pandey, J.; Anand, N.; Tripathi, R. P. *Tetrahedron* 65, **2009**, 9350.
23. (a) Sereda, G. A.; Rajpara, V. B.; Slaba, R. L. *Tetrahedron* 63, **2007,** 8351.
   (b) Sereda, G. A. *Tetrahedron Lett.* 45, **2004**, 7265.
24. Kodomari, M.; Suzuki,Y.; Yoshida, K. *Chem. Commun.* **1997,** 1567.
25. Besson, T.; Thiery, V.; Dubac, J. *Microwaves in Organic Synthesis* 2nd ed.; Wiley-VCH: Weinheim, Germany, **2006;** Vol. 1, pp 416.
26. Sharghi, H.; Sarvari, M. H. *Synthesis,* **2003,** 243
27. Kadam, H. K.; Khan, S.; Kunkalkar, R. A.; Tilve, S. G. *Tetrahedron Lett.* 54, **2013**, 1003.
28. Kassaee, Z. M.; Masrouri, H.; Movahedi, F.; Mohammadi, R. *Helv. Chim. Acta* 93, **2010,** 261.
29. Moghaddas, M.; Davoodnia, A.; Heravi, M. M.; Tavakoli-Hoseini, N. *Chin. J. Catal.* 33, **2012,** 706.
30. (a) Rameshwar, N.; Parthasarathy, T.; Ramreddy, A. *Indian J. Chem.* 47B, **2008,** 1871.
   (b) Ding, D.; Zhao, C-G. *Eur. J. Org. Chem.* **2010**, 3802.
31. (a) Tajbakhsh, M.; Mohajerani, B.; Heravi, M. M.; Ahmadi, N. A. *J. Mol. Catal. A. Chem.* 236, **2005,** 216.
32. Lin, H. X.; Zhao, Q. J.; Xu, B.; Wang, X. H. *Chin. Chem. Lett.* 18, **2007,** 502.
33. (a) Salehi, P.; Dabiri, M.; Zolfigol, A. M.; Fard, B. A. M. *Tetrahedron. Lett.* 44, **2003,** 2889.
   (b) Makaev, F.; Styngach, E.; Muntyanu, V.; Pogrebnoi, S.; Rybkovskaya, Z.; Barba, A. *Russ. J. Org. Chem.* 43, **2007**, 1512.
34. (a) Russowsky, D.; Lopes, F. A.; da Silva, S. S.; Canto, K. F. S.; Montes D' Oca, M. G.; Godoi, M. N. *J. Braz. Chem. Soc.* 15, **2004,** 165.
   (b) da Silvaa, D. L.; Fernandes, S. A.; Sabinoa, A. A.; de Fátimaa A. *Tetrahedron Lett.* 52,**2011**, 6328.
35. Representative procedure for the graphite catalyzed synthesis of 3,4-dihydropyrimidinone/thiones: A mixture of benzaldehyde (106 mg, 1 mmol), urea (60 mg, 1 mmol), ethylacetoacetate (130 mg, 1 mmol) and (10 mg, 10% w/w) graphite was heated at 70°C (120 °C in case of thiourea). The heterogenous mixture slowly became clear and a solid product started to seperate out. After completion of the reaction (1h, TLC) the entire mass solidified. The solid mass was crushed, washed with 5 mL of cold water to remove unreacted urea and filtered.The solid was then dissolved in hot ethanol, and the catalyst was separated by filtration. On cooling the filtrate pure crystals of the product (**1a**) was obtained, yield 97% (237 mg). In all the cases, the product obtained was characterized by comparing spectral data and melting points with literature data.
   Ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate **1a.** white solid, mp 200-202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.008 (t, 3H, *J* = 6.8 Hz, CH_3), 2.182 (s, 3H, CH_3), 3.898 (q, 2H, *J* = 6.8 Hz, CH_2), 5.194 (d, 1H, CH), 6.633(s br., 1H, NH), 7.184-7.081 (m, 5H, aromatic), 8.664 (s br., 1H, NH); ^{13}C NMR (100 MHz, DMSO-d6) δ = 14.07 (CH3), 18.33 (CH_3), 55.15 (CH), 59.63 (CH_2), 100.47 (Cq), 126.59 (2xCH), 127.47 (CH), 128.36(2xCH), 144.39(Cq), 147.37(Cq), 153.24(Cq)165.80 (Cq).ν_{max}(KBr) 3244, 3115, 2980, 1726, 1701, 1649, 1222 cm^{-1}
36. The α-amylase inhibitory study was modified from Miller's method.[37]A solution of α-amylase (ex-porcine pancreas, SRL SiscoPvt Ltd, India) was prepared by mixing 1.28 mg of enzyme in 25 ml of phosphate buffer saline. Synthetic compounds (at a concentration of 50, 150 or 300 µg/ml resuspended in methanol) were added to 15 µl of α-amylase and incubated at 37°C for 30 minutes. After the pre-incubation, 0.5 ml of PBS containing 1% of starch was added and incubated at 37°C for 10 minutes. The reaction was terminated by adding 1 ml of DNSA (3,5-dinitrosalicylic acid) and heating in a boiling water bath for 10 minutes. The tubes were cooled to room temperature and OD at 540 nm was determined using Shimadzu spectrometer. 0.2% (w/v) maltose was used as reference sugar. Acarbose (PHR1253, Fluka) was used as a positive control for demonstrating α-amylase inhibition. All samples were evaluated in triplicates and standard deviation was calculated. Results were expressed as % inhibition = (Test control- test sample/ Test control) × 100.
37. Miller, G. L. *Anal. Chem.* 31, **1959**, 426.

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